

REMARKS

Upon entry of the present amendment, claims 12, 27, 28, 30, 32, and 33-37 are pending in the application. Claims 32 and 33 have been amended herein. Support for these amendments can be found in the originally filed application at, *e.g.*, page 11, lines 20-24 and figure 1. No new matter has been added.

Objections to the Specification and Claims

Applicants acknowledge with appreciation that the Examiner has withdrawn the objections to the specification and claims 12, 26, 29, 32 and 33.

Rejections under 35 U.S.C. § 112, first and second paragraphs

Applicants acknowledge with appreciation that the Examiner has withdrawn the 35 U.S.C. § 112, first and second paragraph rejections of the pending claims.

Rejections under 35 U.S.C. § 102(b)

A. Bentos

Claims 32 and 33 were rejected under 35 U.S.C. § 102(b), as being anticipated by Bentos *et al.* (28 August 1998; “Bentos”). Claims 32 and 33 have been amended. Applicants traverse this rejection to the extent that it applies to the amended claims.

Claim 32 has been amended to require that the isolated nucleic acid molecule hybridizes under stringent conditions to the nucleic acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, and nucleotides 1-600 of SEQ ID NO: 1 under stringent conditions. The nucleic acid disclosed by Bentos is identical to SEQ ID NO: 1 from nucleotides 786 to 941, according to the Examiner in the prior Office action, and is not homologous to either SEQ ID NO: 2 or 3. Thus, Bentos cannot anticipate amended claim 32. Claim 33 has been amended in a similar manner, to require either a) a nucleic acid molecule comprising at least 200 consecutive nucleotides of SEQ ID NO:1, or b) a nucleic acid molecule which hybridizes under stringent condition to nucleotides 1-600 of SEQ ID NO: 2. Bentos is only homologous to SEQ

ID NO: 1 over a span of 155 consecutive nucleotides, from nucleotides 786 to 941 of SEQ ID NO: 1. Thus, Bentos cannot anticipate amended claim 33.

Applicants assert, for the reasons discussed above, that Bentos does not anticipate claims 32 and 33 as amended. Applicants request that this rejection be withdrawn.

B. Ford

Claims 32 -37 were rejected under 35 U.S.C. § 102(b), as being anticipated by Ford (US Patent 6,294,655; "Ford"). Independent Claims 32 and 33 have been amended. Applicants traverse this rejection to the extent that it applies to the amended claims.

According to the Examiner, Ford discloses a sequence that is 86.6% identical to SEQ ID NO:1. However, the region of homology between SEQ ID NO: 1 and the nucleic acid sequence disclosed by Ford begins at nucleotide 271 of SEQ ID NO: 1. Amended claim 32 recites, in part, that the claimed isolated nucleic acid molecule hybridizes under stringent conditions to the nucleic acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, and nucleotides 1-600 of SEQ ID NO: 1. Thus, the homology between the claimed region of SEQ ID NO: 1 and the nucleic acid sequence disclosed by Ford is only 47.6%. Applicants assert that one of skill in the art would recognize that two nucleic acids sharing only 47.6% homology over 600 nucleotides would not hybridize under stringent conditions. Thus, Ford does not anticipate amended claim 32.

Claim 33 has been amended to require either a nucleic acid molecule comprising at least 300 consecutive nucleotides of nucleotides 1-600 of SEQ ID NO:1 or a nucleic acid molecule which hybridizes under stringent condition to nucleotides 1-600 of SEQ ID NO: 1. The nucleic acid alignment between SEQ ID NO :1 of the present application and SEQ ID NO: 8 of Ford provided by the Examiner in the previous Office action demonstrates that the Ford nucleic acid does not meet either limitation. Therefore, Ford cannot anticipate amended claim 33. Applicants request that this rejection be withdrawn.

Rejections under 35 U.S.C. § 101

Claims 12, 27-28, 30 and 32 -37 have been rejected under 35 U.S.C. § 101 for lack of utility. The Examiner has asserted that "the IL-1L-1 polypeptide does not have an activity similar to IL-1 or IL-ra, and since the specification discloses no information regarding the

physiological significance, functional characteristics, or any conditions that involve the nucleic acids of SEQ ID Nos:1, 2, or 3, or the encoded polypeptide, the claimed invention lacks specific and substantial utility or a well-established utility.” (See paragraph bridging pages 6 to 7 of the Office Action). Applicants traverse.

Applicants respectfully assert that the nucleic acids of the present invention have a specific, substantial, and credible utility, and therefore are patentable under 35 U.S.C. §101. As disclosed in the specification, the nucleic acids of the present invention are novel human and murine Interleukin-1 family members found within the IL-1 locus of human chromosome 2, which have sequence homologies with the interleukin-1 receptor antagonist protein (IL-1ra) and interleukin-1 (IL-1). (See, *e.g.*, Figures 5 and 13.) These novel proteins and nucleic acids are referred to in the specification as “IL-1L1.” The IL-1L1 nucleic acid and polypeptide are also known by the following synonyms: IL-1F5, IL-1F8, Interleukin-1 delta, IL-1HY1, IL-1ra homology 1, IL-1 related protein 3, etc. (See, *e.g.*, Swiss-Prot accession number Q9UBH0 available at <http://www.expasy.org/cgi-bin/niceprot.pl?Q9UBH0>, last accessed December 21, 2004.) (Exhibit A) As demonstrated below, the originally filed specification demonstrates that the claimed IL-1L1 nucleic acids, and the polypeptides encoded thereby specific and substantial utility, or a well-established utility.

The IL-1L1 nucleic acid is highly expressed in placenta and, to a lesser extent, in thymus tissues. (See, Figure 7). The expression in the placenta led the inventors to perform a detailed study of the ability of one form (Form B) of the IL-1L1 polypeptide to stimulate IL-6 production using fibroblasts and endothelial cells. (See, Specification at page 120, lines 24-25). However, it has been subsequently determined by others in the field that the IL-1L1 polypeptide, while expressed in keratinocytes, is not expressed in fibroblasts or endothelial cells. (See, *e.g.*, Debets et al., J. Immunol. 167:1440-46 (2001) (“Debets”) (Exhibit B), wherein IL-1L1 is referred to as IL-1F8; courtesy copy provided herewith). This expression pattern explains the inability to measure the effects of IL-1L1 on IL-1 signaling pathways. It has been determined that IL-1L1 does not act through classic IL-1 receptors (IL-1Rs) to initiate IL-1 signaling, such as NF- κ B activation. Rather, IL-1L1 acts by antagonizing the IL-1R6 response to IL-1 ϵ . (See, Debets, pages 1442-43 and Figure 4). Thus, contrary to the Examiner’s assertion, the IL-1L1 peptide of the instant invention has a specific IL-1 antagonistic activity. Therefore, Applicants assert that

the present invention has a specific, substantial, and credible utility in the treatment of diseases or disorders that are associated with an aberrant IL-1L1 level or activity.

Further, Applicant's disagree with the Examiner's assertion that the specification "discloses no information regarding ... any conditions that involve the nucleic acids of SEQ ID Nos: 1-3." The originally filed application discloses that the claimed nucleic acids are useful in diagnostic and prognostic assays to determine if a subject has or is at risk of developing a disease. Non-limiting examples of such diseases are psoriasis and alopecia areata. (See, Specification at page 87, lines 4-27). Moreover, the claimed nucleic acids are useful in screening methods for identifying IL-1L1 therapeutics, *e.g.*, for treating and/or preventing the development of diseases or conditions caused by, or contributed to by an abnormal IL-1L1 activity or which can benefit from a modulation of an IL-1L1 activity or protein level. (See, Specification at page 77, line 17 to page 78, line 6) IL-1L1 is substantially upregulated in lesional psoriasis skin. (See, Debets, pages 1443-44 and Figure 6). Also, genetic analysis of the IL-1L1 gene has demonstrated a strong association of alopecia areata with the IL-1L1 gene and IL-1RN (the gene encoding the IL-1ra polypeptide), and suggests a genetic interaction between these two IL-1 family members. (See, Tazi-Ahnini *et al.*, Eur. J. Immunogenet. 29:25-30 (2002) (Exhibit C)).

Thus, the claimed IL-1L1 nucleic acids are also useful in diagnostic and prognostic assays, and screening methods for identifying IL-1L1 therapeutics. Therefore, Applicants assert that the present invention has a specific, substantial, and credible utility and request withdrawal of this rejection.

Rejection under 35 U.S.C. § 112, first paragraph

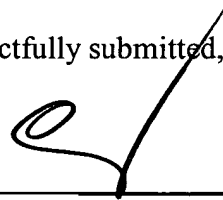
The Examiner has rejected claims 12, 27-28, 30 and 32 -37 under 35 U.S.C. § 112, first paragraph, for not being supported by either a specific or substantial asserted utility. Applicants have demonstrated above, that these claims are supported by such a utility. Therefore, this rejection should be withdrawn.

Applicants: Nicklin *et al.*
U.S.S.N. 09/617,720

CONCLUSION

Applicants submit that the application is in condition for allowance and such action is respectfully requested. Should any questions or issues arise concerning the application, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,



Ivor R. Elrifi, Reg. No. 39,529
Cynthia A. Kozakiewicz, Reg. No. 42,764
Attorneys for Applicant
MINTZ, LEVIN, COHN, FERRIS
GLOVSKY and POPEO, P.C.
One Financial Center
Boston, Massachusetts 02111
Tel: (617) 542-6000

Dated: December 23, 2004

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[\[Features\]](#) [\[Sequence\]](#) [\[Tools\]](#)

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name **I1F5_HUMAN**
Primary accession number **Q9UBH0**
Secondary accession numbers None
Entered in Swiss-Prot in Release 41, February 2003
Sequence was last modified in Release 41, February 2003
Annotations were last modified in Release 46, January 2005

Name and origin of the protein

Protein name **Interleukin 1 family member 5**
Synonyms **IL-1F5**
Interleukin-1 delta
IL-1 delta
FIL1 delta
Interleukin-1-like protein 1
IL-1L1
Interleukin-1 HY1
IL-1HY1
Interleukin-1 receptor antagonist homolog 1
IL-1ra homolog 1
IL-1 related protein 3
IL-1RP3
UNQ1896/PRO4342

Gene name **Name: IL1F5**
Synonyms: FIL1D, IL1HY1, IL1L1, IL1RP3

From Homo sapiens (Human) [TaxID: 9606]

Taxonomy Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.

References

- [1] NUCLEOTIDE SEQUENCE.
TISSUE=Placenta;
DOI=10.1074/jbc.275.2.1169;MEDLINE=20092888;PubMed=10625660 [NCBI, ExPASy, EBI, Israel, Japan]

Smith D.E., Renshaw B.R., Ketchem R.R., Kubin M., Garka K.E., Sims J.E.;
"Four new members expand the IL-1 superfamily."
J. Biol. Chem. 275:1169-1175(2000).

[2] NUCLEOTIDE SEQUENCE.

TISSUE=Fetal skin;
DOI=10.1006/bbrc.1999.1440;MEDLINE=99443727;PubMed=10512743 [NCBI, ExPASy, EBI, Israel, Japan]
Mulero J.J., Pace A.M., Nelken S.T., Loeb D.B., Correa T.R., Drmanac R., Ford J.E.;
"IL1HY1: a novel interleukin-1 receptor antagonist gene."
Biochem. Biophys. Res. Commun. 263:702-706(1999).

[3] NUCLEOTIDE SEQUENCE.

TISSUE=Placenta;
DOI=10.1002/1521-4141(200011)30:11<3299::AID-IMMU3299>3.0.CO;2-S;MEDLINE=20545212;PubMed=11093146 [NCBI, ExPASy, EBI, Israel, Japan]
Barton J.L., Herbst R., Bosisio D., Higgins L., Nicklin M.J.H.;
"A tissue specific IL-1 receptor antagonist homolog from the IL-1 cluster lacks IL-1, IL-1ra, IL-18 and IL-18 antagonist activities."
Eur. J. Immunol. 30:3299-3308(2000).


[4] NUCLEOTIDE SEQUENCE.

MEDLINE=21359532;PubMed=11466363 [NCBI, ExPASy, EBI, Israel, Japan]
Debets R., Timans J.C., Homey B., Zurawski S., Sana T.R., Lo S., Wagner J., Edwards G., Clifford T., Menon S., Bazan J.F., Kastelein R.A.;
"Two novel IL-1 family members, IL-1 delta and IL-1 epsilon, function as an antagonist and agonist of NF-kappa B activation through the orphan IL-1 receptor-related protein 2."
J. Immunol. 167:1440-1446(2001).


[5] NUCLEOTIDE SEQUENCE.

DOI=10.1006/geno.2000.6184;MEDLINE=20318623;PubMed=10860666 [NCBI, ExPASy, EBI, Israel, Japan]
Busfield S.J., Comrack C.A., Yu G., Chickering T.W., Smutko J.S., Zhou H., Leiby K.R., Holmgren L.M., Gearing D.P., Pan Y.;
"Identification and gene organization of three novel members of the IL-1 family on human chromosome 2."
Genomics 66:213-216(2000).

[6] NUCLEOTIDE SEQUENCE [LARGE SCALE MRNA].

DOI=10.1101/gr.1293003;MEDLINE=22887296;PubMed=12975309 [NCBI, ExPASy, EBI, Israel, Japan]
Clark H.F., Gurney A.L., Abaya E., Baker K., Baldwin D.T., Brush J., Chen J., Chow B., Chui C., Crowley C., Currell B., Deuel B., Dowd P., Eaton D., Foster J.S., Grimaldi C., Gu Q., Hass P.E., Heldens S., , Gray A.M.;
"The secreted protein discovery initiative (SPDI), a large-scale effort to identify novel human secreted and transmembrane proteins: a bioinformatics assessment."
Genome Res. 13:2265-2270(2003).

[7] NUCLEOTIDE SEQUENCE.

TISSUE=Placenta;
DOI=10.1073/pnas.242603899;MEDLINE=22388257;PubMed=12477932 [NCBI, ExPASy, EBI, Israel, Japan]
Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G., Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D., Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K., Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., , Marra M.A.;
"Generation and initial analysis of more than 15,000 full-length human and mouse cDNA

sequences.";

Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).

Comments

- **FUNCTION:** Is a highly and a specific antagonist of the IL-1 receptor-related protein 2-mediated response to interleukin 1 family member 9 (IL1F9). Could constitute part of an independent signaling system analogous to interleukin-1 alpha (IL-1A), beta (IL-1B) receptor agonist and interleukin-1 receptor type I (IL-1R1), that is present in epithelial barriers and takes part in local inflammatory response.
- **SUBCELLULAR LOCATION:** Secreted.
- **TISSUE SPECIFICITY:** Predominantly expressed in keratinocytes but not in fibroblasts, endothelial cells or melanocytes. Detected also in the spleen, brain leukocyte and macrophage cell types. Increased in lesional psoriasis skin.
- **INDUCTION:** By phorbol ester (PMA) and lypopolysaccharide (LPS) treatment in macrophage cell line.
- **SIMILARITY:** Belongs to the IL-1 family.

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Cross-references

EMBL	AF201830; AAF25210.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
	AF186094; AAF02757.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
	AJ242737; CAB59822.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
	AJ242738; CAB59823.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
	AJ271338; CAB67704.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
	AF216693; AAF76981.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
	AF230377; AAF91274.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
	AY359117; AAQ89475.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
	BC024747; AAH24747.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence]
PIR	JC7104; JC7104.
HSSP	P18510; 1ILR. [HSSP ENTRY / PDB]
Genew	HGNC:15561; IL1F5.
CleanEx	HGNC:15561; IL1F5.
GeneCards	IL1F5.
GeneLynx	IL1F5; Homo sapiens.
GenAtlas	IL1F5.
MIM	605507 [NCBI / EBI].
GO	GO:0005152; Molecular function: interleukin-1 receptor antagonist activity (traceable author statement).
	QuickGo view.
SOURCE	IL1F5; Homo sapiens.
Ensembl	Q9UBH0; Homo sapiens. [Entry / Contig view]
InterPro	IPR008996; Cytok_IL1_like.
	IPR000975; Interleukin_1. Graphical view of domain structure.
Pfam	PF00340; IL1; 1.

Pfam graphical view of domain structure.
PRINTS PR00264; INTERLEUKIN1.
ProDom PD002536; Interleukin_1; 1.
 [Domain structure / List of seq. sharing at least 1 domain]
SMART SM00125; IL1; 1.
PROSITE PS00253; INTERLEUKIN_1; 1.
HOVERGEN [Family / Alignment / Tree]
BLOCKS Q9UBH0.
ProtoNet Q9UBH0.
ProtoMap Q9UBH0.
PRESAGE Q9UBH0.
DIP Q9UBH0.
ModBase Q9UBH0.
SMR Q9UBH0; B96DB5EFA2612E25.
SWISS-2DPAGE Get region on 2D PAGE.
UniRef View cluster of proteins with at least 50% / 90% identity.

Keywords**Cytokine; Multigene family.****Features**

None

Sequence information

Length: **155** Molecular weight: **16962** CRC64: **B96DB5EFA2612E25** [This is a checksum on the
AA **Da** sequence]

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      70      80      90     100     110     120
VQGGSQCLSC GVGQEPTLTL EPVNIMELYL GAKESKSFTF YRRDMGLTSS FESAAYPGWF

     130     140     150
LCTVPEADQP VRLTQLPENG GWNAPITDFY FQQCD

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Two Novel IL-1 Family Members, IL-1 δ and IL-1 ϵ , Function as an Antagonist and Agonist of NF- κ B Activation Through the Orphan IL-1 Receptor-Related Protein 2¹

Reno Debets, Jackie C. Timans, Bernhard Homey, Sandra Zurawski, Theodore R. Sana, Sylvia Lo, Janet Wagner, Gina Edwards, Teresa Clifford, Satish Menon, J. Fernando Bazan, and Robert A. Kastelein²

IL-1 is of utmost importance in the host response to immunological challenges. We identified and functionally characterized two novel IL-1 ligands termed IL-1 δ and IL-1 ϵ . Northern blot analyses show that these IL-1s are highly abundant in embryonic tissue and tissues containing epithelial cells (i.e., skin, lung, and stomach). In extension, quantitative real-time PCR revealed that of human skin-derived cells, only keratinocytes but not fibroblasts, endothelial cells, or melanocytes express IL-1 δ and ϵ . Levels of keratinocyte IL-1 δ are ~10-fold higher than those of IL-1 ϵ . In vitro stimulation of keratinocytes with IL-1 β /TNF- α significantly up-regulates the expression of IL-1 ϵ mRNA, and to a lesser extent of IL-1 δ mRNA. In NF- κ B-luciferase reporter assays, we demonstrated that IL-1 δ and ϵ proteins do not initiate a functional response via classical IL-1R pairs, which confer responsiveness to IL-1 α and β or IL-18. However, IL-1 ϵ activates NF- κ B through the orphan IL-1R-related protein 2 (IL-1Rrp2), whereas IL-1 δ , which shows striking homology to IL-1 receptor antagonist, specifically and potently inhibits this IL-1 ϵ response. In lesional psoriasis skin, characterized by chronic cutaneous inflammation, the mRNA expression of both IL-1 ligands as well as IL-1Rrp2 are increased relative to normal healthy skin. In total, IL-1 δ and ϵ and IL-1Rrp2 may constitute an independent signaling system, analogous to IL-1 α β /receptor agonist and IL-1R1, that is present in epithelial barriers of our body and takes part in local inflammatory responses. *The Journal of Immunology*, 2001, 167: 1440–1446.

Interleukin 1 family members are known to alter the host response to an inflammatory, infectious, or immunological challenge (1). The biological activity of IL-1 is tightly controlled under physiological conditions. The classical IL-1 family comprises several ligands (i.e., IL-1 α , IL-1 β , and IL-1 receptor antagonist (IL-1ra)³ (2–4), and surface and soluble IL-1 receptors (IL-1RI, IL-1RII, and IL-1R accessory protein (5–7), termed IL-1R1, IL-1R2, and IL-1R3 in this paper, respectively, in keeping with our previously proposed numbering system (8). IL-1 signaling is initiated by high-affinity binding of IL-1 α and β to IL-1R1, which gets subsequently bound by IL-1R3 (5, 7). This results in an intracellular signaling cascade quite similar to stress-induced signal transduction (9), with the end effect being activation of NF- κ B (10). IL-1ra and IL-1R2 antagonize the response to IL-1 α and β at the ligand and (co)receptor levels (2, 3, 11, 12). Numerous studies have shown that perturbation of such control contributes to the pathogenesis of inflammatory and immunological diseases (i.e., leukemia, rheumatoid arthritis, and psoriasis; Refs. 13 and 14).

Recently, new members of the IL-1 family were identified based on both sequence homology and the presence of key structural patterns. For example, IL-18 (15, 16) is predicted to fold as a

β -rich trefoil, typical for IL-1 ligands (17). Moreover, with respect to processing, receptor usage, and signaling, IL-18 can be classified as an IL-1 family member (i.e., IL-1 γ ; Refs. 18–23). In addition, new IL-1 ligands have been identified that show strong structural similarities to IL-1ra (24–29). To date, the expression and especially the receptor usage and function of these IL-1ra-like IL-1 ligands have only been characterized to a limited extent. At the IL-1 receptor level, there also exist additional IL-1R-like molecules. Many of these molecules are currently orphan receptors, such as T1/ST2 (termed IL-1R4; Refs. 30 and 31), IL-1R-related proteins 1 (IL-1R5; Ref. 32) and 2 (IL-1R6; Ref. 33), IL-1R accessory protein ligand (IL-1R7; Ref. 18), single Ig domain IL-1R-related protein (IL-1R8; Ref. 34), IL-1R accessory protein-like (IL-1R9) (35), and IL-1R10 (36), all harboring extracellular Ig-folds and an intracellular domain homologous to the cytosolic part of the *Drosophila* Toll protein. It is interesting to note that the majority of the IL-1 ligands (i.e., IL-1 α β /ra and some IL-1ra-like IL-1 ligands) and IL-1Rs (i.e., IL-1R1, IL-1R2, IL-1R4, IL-1R5, IL-1R6, and IL-1R7) are clustered and localized to chromosome 2 (18, 25, 28, 37–40).

The presence of several orphan IL-1Rs suggests the existence of additional corresponding IL-1 ligands. In line with recent reports (24–28), we have independently identified two novel IL-1 ligands based on sequence homology with IL-1ra, which we termed IL-1 δ and IL-1 ϵ . IL-1 δ corresponds to the reported sequences of IL1H1 (24), FIL1 δ (25), murine IL1H3 (26), IL-1RP3 (27) and IL-1L (28), whereas IL-1 ϵ corresponds to IL1H1 (26) and IL-1RP2 (27). These novel IL-1s are strongly expressed in embryonic tissue and epithelial cells, such as skin keratinocytes. The expression of IL-1 ϵ , and to a lesser extent of IL-1 δ , is significantly up-regulated in IL-1 β /TNF- α -stimulated human keratinocytes. Human IL-1 δ and IL-1 ϵ proteins do not activate NF- κ B through the classical IL-1Rs,

DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304
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¹ DNAX Research Institute is supported by Schering Plough.

² Address correspondence and reprint requests to Dr. Robert A. Kastelein, DNAX Research Institute of Molecular and Cellular Biology, 901 California Avenue, Palo Alto, CA 94304. E-mail address: Kastelein@dnax.org

³ Abbreviations used in this paper: IL-1ra, IL-1 receptor antagonist; EST, expressed sequence tag.

i.e., the IL-1Rs used by IL-1 α and β (IL-1R1 and IL-1R3) or IL-18 (IL-1R5/IL-1R7). Instead, IL-1 ϵ activates this transcription factor via IL-1R6, and this response is potently and specifically antagonized by IL-18. Lesional psoriasis skin shows a substantially increased expression of both the IL-1 ligands as well as their IL-1R. IL-18 and ϵ and IL-1R6 probably constitute an independent signaling system, present in epithelial barriers of our body, which may take part in local inflammatory responses.

Materials and Methods

Biological reagents and cell culture

Recombinant human IL-1 α , IL-1 β , IL-4, IFN- γ , and TNF- α were provided by R&D Systems (Minneapolis, MN). Recombinant human IL-18 and IL-1 ϵ were produced at DNAX Research Institute of Molecular and Cellular Biology (Palo Alto, CA). The Q293 and 293-T cell lines were maintained in DMEM supplemented with 5% FBS, 0.3 mg/ml L-glutamine, 100 U/ml penicillin G, and 100 μ g/ml streptomycin (Life Technologies, Paisley, U.K.). Human primary epidermal keratinocytes, dermal fibroblasts, dermal microvascular endothelial cells, and melanocytes (Clonetics, San Diego, CA) were cultured in specialized growth medium according to the suppliers' recommendations. The Jurkat E6.1 cell line was maintained in RPMI 1640 medium supplemented with 10% FBS, glutamine, and antibiotics.

Cloning of human and mouse IL-18 and IL-1 ϵ

BLAST searches in the public mouse expressed sequence tag (EST) database with the common portion of murine IL-1 α revealed EST mb49b11.r1 (GenBank accession no. W08205). The insert contained the full-length sequence of a novel IL-1-like molecule, designated IL-18. With this mouse sequence as a query, a human EST (5120028H1), derived from RNA from bronchial smooth muscle cells, was found in our proprietary Incyte database (Palo Alto, CA) that contained the full-length open reading frame of the human ortholog of mouse IL-18. The same query sequence revealed an additional EST mi08c10.r1 in the public mouse database (GenBank accession no. AA030324), which contained partial sequence of a second novel IL-1-like molecule, designated IL-1 ϵ . The full-length sequence of murine IL-1 ϵ was obtained by extending the 5' sequence by PCR on murine 17-day-old embryo Marathon-Ready library cDNA (Clontech, Palo Alto, CA). Separately, a Hidden Markov Model HMMer search (<http://hmm.wustl.edu/>) with a PFAM alignment of IL-1 α , IL-1 β , and IL-1 ϵ (<http://pfam.wustl.edu/>) revealed an EST (HAICR08) derived from RNA from epithelial cells in the Human Genome Sciences database (Rockville, MD) that contained the full-length open reading frame of human IL-1 ϵ .

A multiple alignment of these novel IL-1 sequences and published IL-1 sequences was created using CLUSTALW (41), guided by tertiary structures and predicted secondary structures (with a consensus derived from several algorithms at <http://circinus.ebi.ac.uk:8081/submit.html>), and refined by eye. Conserved alignment patterns were drawn by Consensus (<http://www.bork.embl-heidelberg.de/Alignment/consensus.html>). Evolutionary tree analysis was performed with a neighbor-joining algorithm and viewed with TreeView 1.5 (<http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>).

Protein expression and purification of human IL-18 and IL-1 ϵ

Adenoviral vectors containing full-length human IL-18 and IL-1 ϵ sequences were constructed by PCR and used to transfect Q293 packaging cells. Viruses were subsequently purified, with all procedures according to the manufacturer's protocols (Invitrogen, Carlsbad, CA). Q293 cells (5×10^8) were infected (adenoviruses used at 10 multiplicity of infection) and incubated for 5 days in a cell factory in a total volume of 1 L of serum-free CMF-1 medium (Life Technologies). Culture medium was dialyzed (Spectra/Por membrane tubing; molecular mass cut-off, 6–8 kDa; Spectrum Laboratories, Rancho Dominguez, CA) against 50 mM Tris-HCl, pH 8.0, and 1 mM EDTA, and subsequently passed through hitrap Q Sepharose and heparin columns. The flow-through, containing the IL-1 proteins, was sterile-filtered and concentrated ~70 times with an Amicon 8400 ultrafiltration cell with a 10-kDa molecular mass cut-off membrane (Millipore, Bedford, MA). The samples were dialyzed against PBS, and the protein content was quantified by PAGE and Coomassie blue staining with lysozyme as a standard. Protein identities were confirmed by N-terminal sequencing. Identically treated culture medium of Q293 cells infected with adenovirus encoding green fluorescent protein served as a negative control. Endotoxin levels were determined by using the *Limulus* amoebocyte lysate assay (Bio-Whittaker, Walkersville, MD) and were <1.5 EU/100 μ g protein. Protein samples were stored at 4°C.

Expression plasmids

Plasmids encoding full-length human R1, mouse R3, mouse R4, human R5, and human R7 sequences were constructed by inserting PCR-generated cDNA fragments into pME18S (42). Human IL-1R6 cDNA, a generous gift of Dr. R.A. Maki (Neurocrine Biosciences, San Diego, CA), was subcloned directly into pME18S. The reporter gene plasmid pNF- κ B-Luc (Stratagene, La Jolla, CA) contains five NF- κ B sites and a basic promoter element to drive luciferase expression, and pRSV- β Gal results in constitutive expression of β -galactosidase.

Northern blots

Mouse Northern blots containing ~2 μ g of poly(A)⁺RNA per lane, derived from either total embryo at different days postgestation (Clontech) or from various adult tissues (Origene Technologies, Rockville, MD), were hybridized to the mouse IL-18 and IL-1 ϵ cDNA probes containing the complete open reading frames. Probes were labeled with ³²P by using the Redivue labeling kit (Amersham Pharmacia Biotech, Uppsala, Sweden). Prehybridization, hybridization, stringency washes, and stripping were conducted according to the manufacturer's protocols. Membranes were exposed to a phosphorimager.

Quantitative real-time PCR

A panel of various human skin-derived cells, i.e., primary epidermal keratinocytes, dermal fibroblasts, dermal microvascular endothelial cells, and melanocytes, as well as skin biopsies and PBMC were used for TaqMan-PCR analyses. Skin-derived cells were left untreated or were treated with IL-4 (50 ng/ml), IFN- γ (20 ng/ml), or a combination of IL-1 β (5 ng/ml) and TNF- α (10 ng/ml) for 18 h before RNA isolation. Biopsies from lesional psoriasis skin and normal healthy skin were kindly donated by Dr. T. Ruzicka (Department of Dermatology, University of Dusseldorf, Germany) and homogenized before RNA isolation. PBMC from a healthy donor, prepared by standard protocols, stimulated with and without PHA served as controls. RNA isolation, cDNA synthesis, and PCR were performed as described elsewhere (43). The amplicons for human IL-18 (nt 17–90, with numbers starting at first methionine codon), human IL-1 ϵ (nt 337–409), and human IL-1R6 (nt 1378–1448) were analyzed with 6-carboxy-fluorescein-labeled probes. 18S RNA quantities were measured with a VIC-labeled probe and served as internal controls to normalize for the total amount of cDNA. Values are expressed as fg/5 ng total cDNA.

Reporter assay

Jurkat E6.1 cells (4×10^6) were transiently transfected with pNF- κ B-Luc reporter gene plasmid, pRSV- β Gal plasmid, and IL-1R-encoding cDNA plasmid(s) as described previously (43). Twenty hours after transfection, cells were stimulated with 20 ng/ml of human IL-1 α , IL-1 β , IL-18, or IL-1 ϵ , or 50 ng/ml human IL-18 or IL-1 ϵ for 6 h. Cells were lysed with reporter lysis buffer (Promega, Madison, WI), and luciferase and β -galactosidase activities were assessed with luciferase assay reagent (Promega) and Galacto-Light Kit (Tropix, Bedford, MA), respectively. Luciferase activities (in RLU) were normalized on the basis of β -galactosidase activities. For inhibition studies of IL-1R1-mediated activation of NF- κ B, IL-1 α was used at 50 pg/ml in the presence of IL-1 α and IL-18 at concentrations ranging from 10 pg/ml to 10 μ g/ml. Inhibition of the IL-1R6-mediated response was analyzed with IL-1 ϵ used at 50 ng/ml and IL-18 or IL-1 α at concentrations ranging from 64 pg/ml to 10 μ g/ml.

Results

IL-18 and ϵ were identified computationally

Computational analyses led to the discovery of two novel IL-1 ligands: IL-18 and IL-1 ϵ . In short, the strategies used both homology-based and probabilistic-based (HMMer) searches (see *Materials and Methods* for details). The sequences, and a comparison with the previously known IL-1 family members, are given in Fig. 1A. Fig. 1B, the corresponding dendrogram, shows evolutionary relationships.

IL-18 and IL-1 ϵ messenger RNA are highly expressed in embryonic tissue and in epithelial cells

Northern blot analyses show that IL-18 and IL-1 ϵ are expressed in embryonic tissue and tissues containing epithelial cells (i.e., stomach and skin; Fig. 2). Quantitative PCR analyses on a large panel of mouse and human tissue cDNAs (including various lymphoid

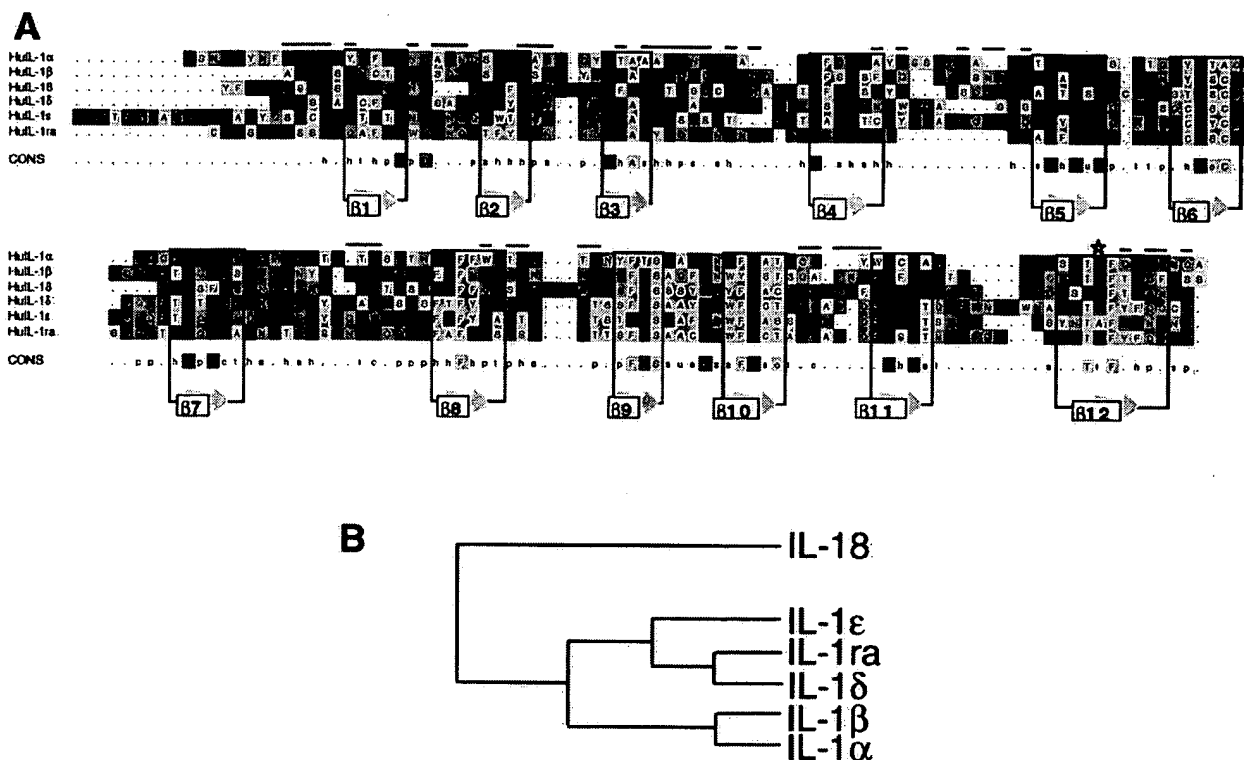


FIGURE 1. Typical IL-1-like structural patterns are conserved in IL-1 δ and IL-1 ϵ . **A**, Sequence alignment of mature IL-1 sequences: human IL-1 α starts at L119 (GenBank accession no. AAA59134), human IL-1 β starts at A117 (AAA59135), human IL-18 starts at Y37 (BAA08706), and human IL-1ra starts at C25 in the common region (CAA36262). Human IL-18 and IL-1 ϵ both start at their first M. The 12 β -strands, typical for IL-1 ligands, are depicted by arrows numbered from β 1 to β 12. The β -strands are boxed according to known (i.e., human IL-1 β and IL-1ra; Refs. 50 and 51) and predicted secondary structures. Lines above the alignment indicate residues of human IL-1 β that interact with IL-1R1 (sites A and B as in (51), in black and red, respectively). The asterisk indicates a residue, which together with the loop region between β 4 and β 5, is crucial in determining antagonist activity in IL-1ra (49, 52). The amino acid coloring scheme depicts chemically similar residues: green (hydrophobic); red (acidic); blue (basic); yellow (C); orange (aromatic); black (structure breaking); and gray (tiny). Diagnostic sequence patterns for IL-1s were derived by CONSENSUS at a stringency of 70%. Symbols for amino acid subsets are as follows: o, alcoholic; l, aliphatic; dot, any amino acid; a, aromatic; c, charged; h, hydrophobic; -, negative; p, polar; +, positive; s, small; u, tiny; and t, turnlike. **B**, Evolutionary dendrogram of IL-1s. Sequence data for human and mouse IL-1 δ are available from GenBank under accession nos. AF230377 and AF230378, respectively; sequence data for human and mouse IL-1 ϵ are available under AF206696 and AF206697, respectively.

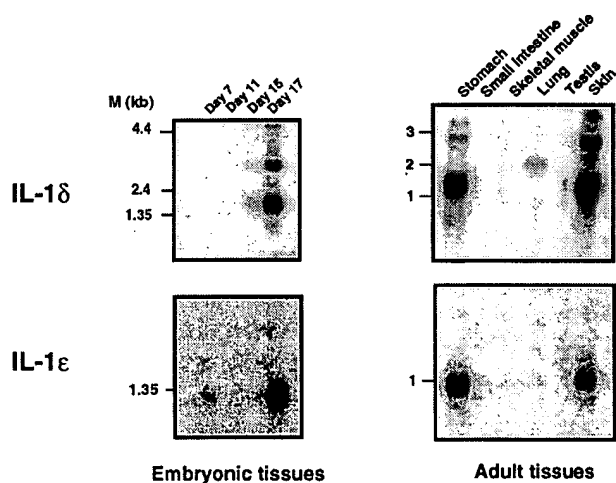


FIGURE 2. IL-1 δ and IL-1 ϵ messenger RNA are highly expressed in embryonic tissue and in tissues containing epithelial cells. Northern blot analyses of mouse IL-1 δ and IL-1 ϵ messenger RNA expression in embryonic tissues at different days postgestation and in different adult tissues. Multiple tissue blots containing $\sim 2 \mu\text{g}$ poly(A)⁺ RNA per lane were hybridized to the IL-1 δ and IL-1 ϵ cDNA probes. Molecular weight RNA sizes in kilobases are indicated. See *Materials and Methods* for details.

organs, kidney, heart, lung, brain, liver, organs of the digestive tract, reproductive organs, and skin) confirmed these findings. Messenger RNA expression in lung tissue appears to be unique to IL-1 δ (Fig. 2). It should also be noted that IL-1 δ mRNA analysis shows the presence of multiple variants. More in-depth studies, based on quantitative PCR, revealed that in skin, keratinocytes but not fibroblasts, endothelial cells, or melanocytes are the main producers of IL-1 δ and IL-1 ϵ (Fig. 3). In vitro-cultured keratinocytes contained ~ 10 -fold more IL-1 δ mRNA relative to IL-1 ϵ mRNA. Stimulation with IL-4 or IFN- γ hardly affected the expression levels of IL-1 δ and ϵ mRNA, whereas stimulation with a combination of IL-1 β and TNF- α resulted in an enormous increase in the expression of IL-1 ϵ mRNA and to a lesser extent of IL-1 δ mRNA (Fig. 3).

IL-1 δ and IL-1 ϵ do not activate NF- κ B through classical IL-1Rs

The ability of the novel IL-1s to initiate IL-1R-mediated signaling was studied via an NF- κ B-dependent reporter assay with ligand-stimulated Jurkat T cells transiently transfected with different pairs of IL-1Rs. The R1/R3 combination, conferring responsiveness to IL-1 α and IL-1 β (5, 44), did not generate a response to IL-1 δ or IL-1 ϵ . Also, the R5/R7 combination, required to mediate a response to IL-18 (18, 43), did not result in signaling on addition of the novel IL-1 ligands (Fig. 4A). The next step was to test the

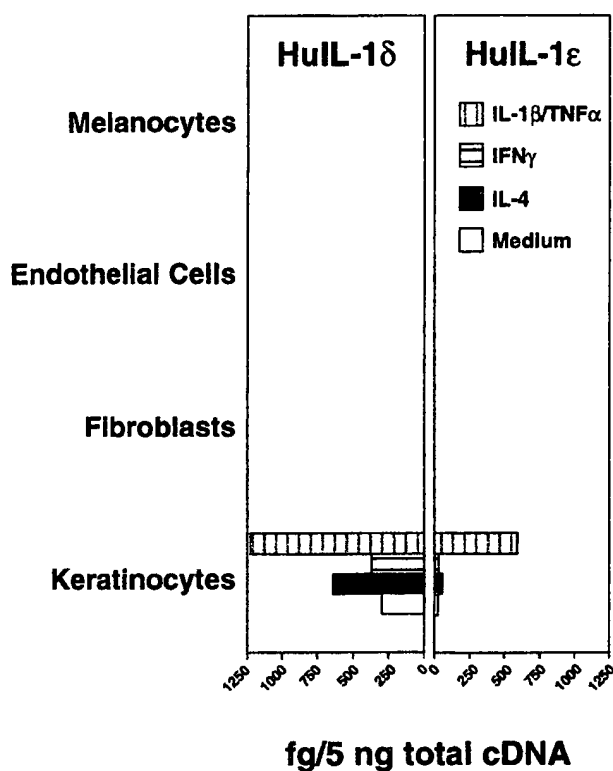


FIGURE 3. Keratinocytes are the main source for IL-1 δ and IL-1 ϵ messenger RNA in skin tissue. A panel of human cDNAs from various skin-derived cells were analyzed for expression levels of human IL-1 δ and IL-1 ϵ by the Fluorogenic 5'-nuclease PCR assay. The novel IL-1s were analyzed by FAM-labeled probes. Expression levels were normalized to reverse-transcribed 18S RNA quantities and expressed as fg/5 ng total cDNA. Cells were left untreated or treated with different cytokines for 18 h as indicated before RNA was isolated. See *Materials and Methods* for details.

orphan IL-1R-like molecules IL-1R4 and IL-1R6 (classified as potential ligand-binding receptors based on their homology to IL-1R1; Ref. 36) paired with various other IL-1R-like molecules, i.e., IL-1R3, IL-1R7, IL-1R9, and IL-1R10 (classified as potential signaling receptors based on their homology to IL-1R3; Ref. 36) for their capacity to confer responsiveness to IL-1 δ and IL-1 ϵ . Data consistently showed an IL-1R6-mediated activation of NF- κ B upon stimulation with IL-1 ϵ , but not IL-1 δ or the mock control (Fig. 4B).

IL-1 δ specifically and very potently antagonizes the IL-1R6 response to IL-1 ϵ

In line with the striking similarity between IL-1 δ and IL-1 α , we tested the possibility of IL-1 δ being an antagonist of IL-1 responses rather than being an IL-1 agonist. With the same reporter assay with IL-1R-transfected Jurkat T cells, we showed that IL-1 α , but not IL-1 δ , is able to antagonize the IL-1R1-mediated activation of NF- κ B on stimulation with IL-1 α (Fig. 5A). Vice versa, IL-1 δ , but not IL-1 α , is able to antagonize the IL-1R6-mediated activation of NF- κ B on stimulation with IL-1 ϵ (Fig. 5B). Importantly, IL-1 α shows a 50% inhibition of the IL-1R1-mediated response to IL-1 α at about a 1000-fold excess over IL-1 α , whereas IL-1 δ results in a similar inhibition of the IL-1R6-mediated response to IL-1 ϵ at concentrations similar to or even less than IL-1 ϵ .

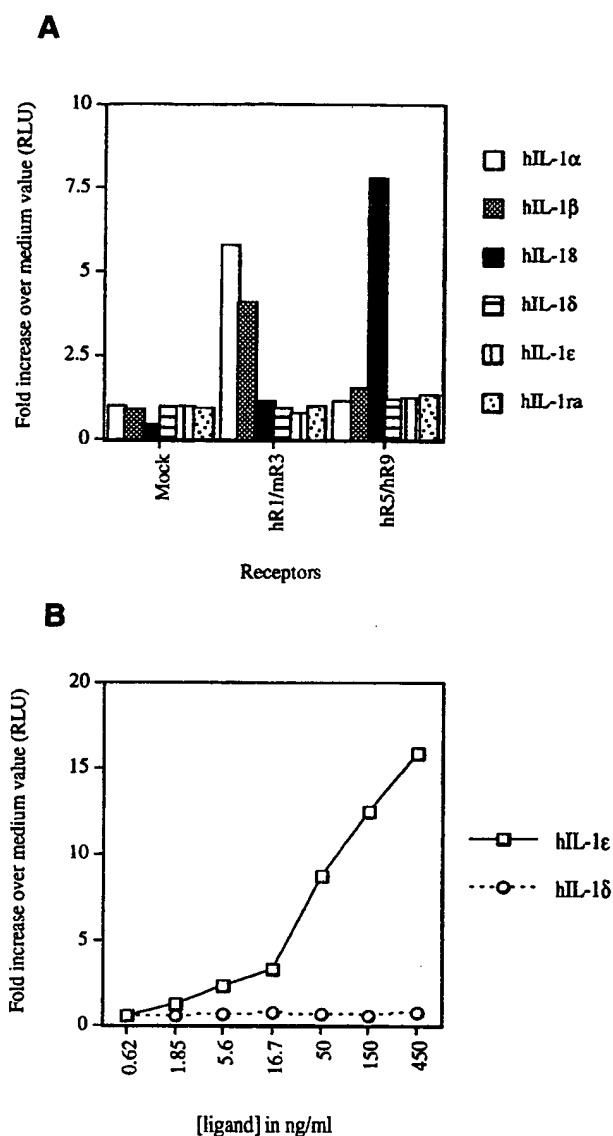


FIGURE 4. A, IL-1 δ and IL-1 ϵ do not activate NF- κ B through classical IL-1Rs. Jurkat cells (4×10^6) were transfected with 2 μ g of pNF- κ B-Luc reporter gene plasmid, 0.5 μ g of pRSV- β Gal plasmid, and 4 μ g of each IL-1R plasmid (in pME18S, all human, except mouse IL-1R3 plasmid) as indicated. Twenty hours after transfection, cells were left untreated or were stimulated for 6 h with human IL-1 ligands (20 ng/ml final, except IL-1 δ or IL-1 ϵ , which is used at 50 ng/ml final). Luciferase activities were determined and normalized on the basis of β -galactosidase activities. Single receptors did not give any luciferase response. Data shown are from one of two independent experiments with similar results. B, IL-1 ϵ , but not IL-1 δ , activates NF- κ B through IL-1R6. See A for details. Jurkat cells were transfected with 0.5 μ g of human IL-1R6 plasmid (in pME18S). Twenty hours after transfection, cells were left untreated or were stimulated for 6 h with human IL-1 δ or IL-1 ϵ at different concentrations. Mock protein control for human IL-1 δ and IL-1 ϵ gave a luciferase response similar to medium only. Data shown are from one of three independent experiments with similar results.

IL-1 δ and ϵ and IL-1R6 levels are substantially up-regulated in lesional psoriasis skin

In lesional psoriasis skin, characterized by chronic cutaneous inflammation, the expression of the novel IL-1 ligands, IL-1 δ and IL-1 ϵ , and IL-1R6 are all significantly increased relative to skin

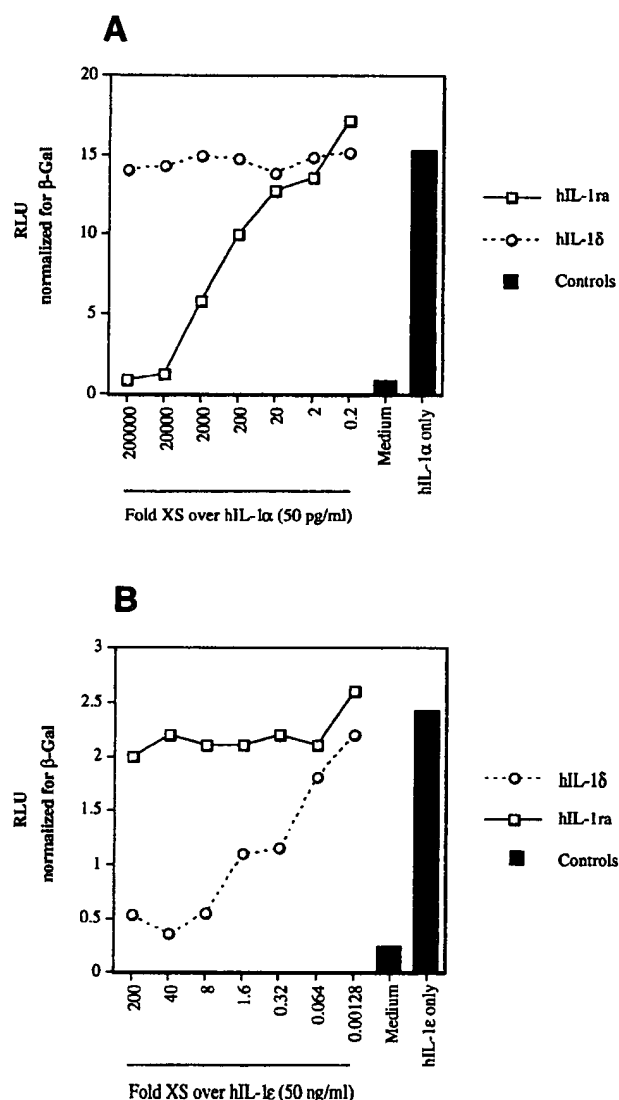


FIGURE 5. IL-1 δ specifically antagonizes the IL-1R6-mediated response to IL-1 ϵ . *A*, IL-1ra, but not IL-1 δ , antagonizes the IL-1R1-mediated response to IL-1 α . See legend to Fig. 4*A* for details. Jurkat cells were transfected with 0.5 μ g of human IL-1R1 plasmid. Twenty hours after transfection, cells were left untreated or were stimulated for 6 h with human IL-1 α at 50 pg/ml final concentration with or without human IL-1ra or IL-1 δ at final concentrations ranging from 10 pg/ml to 10 μ g/ml. Antagonist only (i.e., IL-1ra or IL-1 δ) and mock protein controls, even at high concentrations, did not give any luciferase response. Data shown are from one of two independent experiments with similar results. *B*, IL-1 δ , but not IL-1ra, antagonizes the IL-1R6-mediated response to IL-1 ϵ . Jurkat cells were transfected with 0.5 μ g of human IL-1R6 plasmid. Twenty hours after transfection, cells were left untreated or were stimulated for 6 h with human IL-1 ϵ at 50 ng/ml final with or without human IL-1 δ or IL-1ra at final concentrations ranging from 64 pg/ml to 10 μ g/ml. Antagonist only and mock protein controls, even at high concentrations, did not give any luciferase response. Data shown are from one of three independent experiments with similar results.

from a healthy individual (Fig. 6). The increase is most prominent for IL-1 ϵ , in line with in vitro-cultured keratinocytes stimulated with the pro-inflammatory cytokines IL-1 β /TNF- α (Fig. 3). Activation of PBMC also leads to increased levels of both IL-1 ligands and their receptor, albeit to a lesser extent than observed in lesional psoriasis skin.

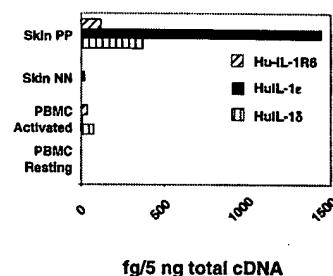


FIGURE 6. Expression of both IL-1 δ and IL-1 ϵ and IL-1R6 are increased in lesional psoriasis skin. Biopsies from lesional psoriasis skin (PP) and normal healthy skin (NN) and PBMC from a healthy donor stimulated with and without PHA were analyzed for expression levels of human IL-1 δ and IL-1R6 by the Fluorogenic 5'-nuclease PCR assay. See Fig. 3 for details.

Discussion

This paper describes the discovery of two novel members of the IL-1 family, termed IL-1 δ and IL-1 ϵ . Several recent studies have reported on the cloning and molecular characterization of IL-1 δ and IL-1 ϵ (24–28). However, the present study is the first to report on the expression in human skin-derived cell types, receptor usage, and initial functional characterization of IL-1 δ and IL-1 ϵ .

The structurally aided alignment of both the novel and classical IL-1s in Fig. 1*A* shows the conservation of the core 12 β -strands, making up the β -trefoil structure. The presented sequence of human IL-1 δ protein is identical with the reported sequences of IL1H1 (24), FIL1 δ (25), IL-1RP3 (27), and IL-1L (28). At the amino acid level, human IL-1 δ and IL-1ra show a high degree of similarity, which is confirmed by the evolutionary tree analysis (Fig. 1*B*). With respect to IL-1 ϵ , the presented sequence is identical with the sequences of IL1H1 (26) and IL-1RP2 (27). It is interesting to note that several public mouse ESTs exist, mostly derived from tongue epithelium, with only slight variations relative to the IL-1 ϵ sequence. In addition, FIL1 ϵ (25) also shows a very high similarity to the human IL-1 ϵ sequence presented in this paper (i.e., 51%). How these IL-1 ϵ variants are generated and their biological significance remain unclear.

IL-1 δ and IL-1 ϵ are strongly expressed in embryonic development and in tissues such as stomach, lung, and skin (Fig. 2 and PCR analyses on a panel of various tissue cDNAs not shown). Lung tissue only showed expression of IL-1 δ messenger RNA (at the Northern blot level), although at the PCR level, expression of both IL-1 δ and IL-1 ϵ can be detected in lung-derived cDNAs. Sizes of the predominant messages for IL-1 δ are 1.4 and 2.7 kb for stomach and skin tissues, respectively, and 2.0 kb for lung tissue. Interestingly, IL-1 δ messenger RNA in lung tissue is reported to lack the second exon relative to other tissues (25). In analogy to IL-1ra, the different IL-1 δ messages might reflect different (tissue-specific) splice variants (2, 45–47). In fact, both IL-1 δ and IL-1ra mRNA sequences diverge at the 5' ends because of usage of alternative first exons (27). A detailed analysis of human skin was performed with quantitative PCR analysis on a panel of first-strand cDNAs derived from various skin-specific cell types. Keratinocytes, but not fibroblasts, endothelial cells, or melanocytes were identified as the major source for IL-1 δ and IL-1 ϵ , with levels of IL-1 δ being ~10-fold higher than those of IL-1 ϵ (Fig. 3). In addition, Langerhans cells but not skin-homing T cells, freshly isolated from skin biopsies, showed some expression of IL-1 δ and ϵ (data not shown). In vitro stimulation of keratinocytes with pro-inflammatory cytokines (i.e., IL-1 β /TNF- α) but not with IL-4 or IFN- γ significantly up-regulated the expression of IL-1 ϵ , and to a

lesser extent of IL-18 (Fig. 3). Our observations are in line with reports on the constitutive and induced expression of keratinocyte IL-18 and IL-1 ϵ mRNA (26, 27). Preliminary in situ hybridization data using mouse tissue sections confirmed that cells of epithelial origin, such as the parietal and chief cells in stomach, and basal keratinocytes in skin are the predominant cellular sources of these IL-1s (not shown). In addition, esophageal squamous epithelium is also reported to express IL-1 ϵ (27). The presence of IL-18 ϵ in epithelial barriers of our body (i.e., skin, digestive, and respiratory tracts), suggests that these novel IL-1s fulfill similar roles as their known family members (i.e., IL-1 α and IL-1 β) to promote a response to injury or infection (1, 48). In fact, Kumar and colleagues have shown that the epidermal expression of murine IL-1 ϵ is up-regulated in vivo in response to contact hypersensitivity or a viral infection (26).

It is important to note that IL-18 and IL-1 ϵ neither possess a classical leader sequence (as does secreted IL-1 α ; Ref. 2) nor do they possess a distinct pro-form (as do IL-1 α β and IL-18; Refs. 4, 15, and 16). However, monitoring the presence of C-terminally tagged versions of IL-18 and IL-1 ϵ in the supernatants and lysates of transfected 293-T cells (human epithelial cells) revealed that these molecules are secreted as 20-kDa proteins (data not shown). This is in agreement with the finding that the human trophoblastic tumor cell line JEG-3 is able to secrete IL-18 (28), and argues that an alternative mechanism exists to secrete these novel IL-1s. To functionally characterize the novel IL-1s, we expressed and purified adenovirally derived human IL-18 and IL-1 ϵ and tested these proteins for their capacity to initiate IL-1 signaling, with NF- κ B activation as a read-out. The observation that IL-1R1/3 and IL-1R5/7 do not respond to these new protein preparations (Fig. 4A) might be explained by the fact that receptor-ligand combinations within the IL-1 system are very specific (43). Therefore, we subsequently tested the orphan receptors IL-1R4 and IL-1R6 paired with various other IL-1R-like molecules. These studies consistently showed that IL-1R6 responded to IL-1 ϵ but not IL-18 in activating NF- κ B in Jurkat cells (Fig. 4B). Even IL-1R6 single transfectants showed this response. The IL-1 system, as we know it today, typically requires two receptors, a ligand-binding subunit and a signaling subunit, to get an IL-1 response (5, 18). Because IL-1R6 is very homologous to IL-1R1 (33), a ligand-binding type of receptor, we believe that Jurkat cells endogenously express a second signaling type of receptor that can pair with IL-1R6 in the presence of IL-1 ϵ . We know that the following IL-1R-like molecules are expressed by nontransfected Jurkat cells: IL-1R3, IL-1R4, IL-1R8, IL-1R9, and IL-1R10 (PCR data, not shown). Co-transfection of IL-1R6 with either IL-1R3, IL-1R9, or IL-1R10 does not potentiate the response to IL-1 ϵ relative to IL-1R6 single transfectants (not shown). In addition, studies by others with IL-1R1 chimeras and IL-1 α -mediated activation of NF- κ B as a read-out do not support a combination of IL-1R6 and IL-1R8 to mediate an IL-1 response (34). The search for the additional IL-1 ϵ receptor(s) is currently ongoing.

IL-18 is most closely related to IL-1 α , and, like IL-1 α , lacks the loop between the fourth and fifth β -strands (see Fig. 1A), which is typical for IL-1 agonists: IL-1 α , IL-1 β , IL-18 and IL-1 ϵ . In fact, insertion of the loop amino acids QGEESN of IL-1 β confers agonist activity to IL-1 α (49). Therefore, we hypothesized that IL-18 acts as an antagonist. Indeed, IL-18 is a very potent antagonist of the IL-1R6-mediated response to IL-1 ϵ at a ratio of IL-18:IL-1 ϵ <1 (Fig. 5). Note that the potency of IL-1 α to antagonize the IL-1R1-mediated response to IL-1 α is \sim 3 orders of magnitude less. The observation by others (25) that their FIL18 and FIL1 ϵ proteins do not bind to IL-1R6 is in our opinion not contradictory to our findings. Binding studies with partially purified IL-18 and

IL-1 ϵ proteins from conditioned medium of transfected cells and an Fc fusion of IL-1R6 might not be sensitive enough to show binding to these new IL-1s. Moreover, the second receptor might actually be needed for affinity conversion and for binding to become detectable (5, 43).

IL-18 is a highly specific antagonist of the IL-1R6-mediated response to IL-1 ϵ . For instance, IL-18 does not respond through IL-1R1, either as an agonist or antagonist (see Figs. 4 and 5), which confirms the reported lack of IL-18 to induce the production of IL-6 or inhibit the IL-1 α β -induced production of IL-6 by cultured fibroblasts or endothelial cells (28). Moreover, IL-18 does not respond through IL-1R5 because IL-18 does not induce the production of IFN- γ or inhibit the IL-18-induced production of IFN- γ by KG-1 cells (28). In fact, a recently cloned IL-1 α homologue, termed IL-1H (with various isoforms: FIL1 ζ (25), IL1H4 (26), and IL-1RP1 (27)) was shown to bind to IL-1R5 but not IL-1R1 (29), and may act as a specific IL-18 antagonist.

Expression of human IL-1R6 is restricted to lung epithelium and brain vasculature (33). In extension to these findings, we observed expression of IL-1R6 mRNA in monocytes and in skin-derived keratinocytes, fibroblasts and to a lesser extent endothelial cells. With respect to skin cells, IL-1R6 may in fact mediate proliferation and production of matrix metalloproteinases in response to IL-1 ϵ (preliminary data, not shown). Activated monocytes also show an up-regulated expression of IL-18 and IL-1 ϵ mRNA that probably explains the presence of these IL-1s in activated PBMC (Fig. 6). The expression of IL-18 and IL-1 ϵ , as well as IL-1R6, mRNA are all, but most notably IL-1 ϵ mRNA, highly increased in lesional psoriasis skin samples relative to normal control skin samples (Fig. 6). These data are momentarily followed up, but already confirm the involvement of these novel IL-1s in response to skin inflammation (26) and extend the notion that IL-1 ligands and receptors contribute to the pathogenesis of psoriasis (13).

Taken together, IL-18 and ϵ and IL-1R6 may constitute an independent signaling system analogous to IL-1 α β /ra and IL-1R1. The IL-1R6 system, present in epithelial barriers of our body, as a result from the coexpression of IL-18 and IL-1 ϵ , may be in a default off-state. However, perturbation of homeostasis can shift this balance to an IL-1 ϵ -mediated inflammatory or proliferative response, as seen in lesional psoriatic skin.

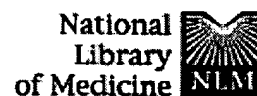
Acknowledgments

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Genetic analysis of the interleukin-1 receptor antagonist and its homologue IL-1L1 in alopecia areata: strong severity association and possible gene interaction.

Tazi-Ahnini R, Cox A, McDonagh AJ, Nicklin MJ, di Giovine FS, Timm JM, Messenger AG, Dimitropoulou P, Duff GW, Cork MJ.

Division of Genomic Medicine, University of Sheffield, Royal Hallamshire Hospital, Sheffield S10 2JF, UK. r.taziahnini@sheffield.ac.uk

Alopecia areata is an inflammatory hair loss disease with a major genetic component. The presence of focal inflammatory lesions with perifollicular T-cell infiltrates reflects the importance of local cytokine production in the pathogenesis. In addition to its fundamental pro-inflammatory role, the interleukin-1 (IL-1) system has major effects on hair growth regulation in vit with the inhibitory actions of IL-1alpha and IL-1beta being opposed by the receptor antagonist IL-1ra. The novel interleukin-1 like molecule 1 (IL-1L1) which has greatest gene sequence homology with IL1RN, the gene encoding 1ra, is another potential IL-1 antagonist. In view of previous studies suggesting a significant role for IL1RN polymorphisms in the pathogenesis of autoimmune/inflammatory disease, we have analysed polymorphisms of IL-1 (IL1RN+2018) and its homologue IL-1L1 (IL1L1+4734) in a case-control association study on 165 patients and a large number of matched controls. Homozygosity for the rare allele of IL1RN (IL1RN*2) was significantly associated with alopecia areata [odds ratio (OR) = 1.89, 95% CI (1.09, 3.28); = 0.02], confirming our previous findings of significant association with the IL1RN variable number tandem repeat (VNTR). The results also revealed a novel association involving a polymorphism of the interleukin-1 receptor antagonist homologue IL1L1 at position + 4734, IL1RN+2018, and alopecia areata. The effect of a genotype combining three copies of the rare alleles at t IL1RN and IL1L1 loci conferred a more than additive increase in the risk of disease compared to IL1RN+2018 or IL1L1+4734 alone [OR 3.37 (1.60, 7.06 P = 0.002], suggesting possible synergy between the IL1RN and IL1L1 genes. This effect was stronger in patients with severe disease (alopecia totalis/universalis) [OR 4.62 (1.87, 11.40), P = 0.0022], and in those with ear age at onset (< 20 years) [OR = 6.38 (2.64, 15.42), P = 0.0002]. Our results suggest that these polymorphisms within IL1RN and IL1L1 themselves or a gene in linkage disequilibrium with IL1RN and IL1L1 predispose to the more

severe forms of alopecia areata.

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